

J. Schöpfung Rehage · Beverly K. Barnett · Andrew Sih

Behavioral responses to a novel predator and competitor of invasive mosquitofish and their non-invasive relatives (*Gambusia* sp.)

Received: 30 June 2004 / Revised: 23 August 2004 / Accepted: 1 September 2004 / Published online: 8 October 2004
© Springer-Verlag 2004

Abstract Attributes of the recipient community may affect the invasion success of arriving non-indigenous organisms. In particular, biotic interactions may enhance the resistance of communities to invasion. Invading organisms typically encounter a novel suite of competitors and predators, and thus their invasiveness may be affected by how they cope with these interactions. Behavioral plasticity may help invaders to respond appropriately to novelty. We examined the behavioral responses of highly invasive mosquitofish to representative novel competitors and predators they might encounter as they spread through North America. We compared the behavior of invasive *Gambusia holbrooki* and *G. affinis* to that of two close relatives of lower invasive potential (*G. geiseri* and *G. hispaniolae*) in order to elucidate whether responses to novelty related to invasiveness. In short-term assays, female *Gambusia* were paired with a novel competitor, *Pimephales promelas*, and a novel predator, *Micropterus dolomieu*. Behavioral responses were measured in terms of foraging success and efficiency, activity, refuge use, predator inspections, and interspecific aggression. Contrary to a priori predictions, invasive and non-invasive responses to novel interactions did not differ consistently. In response to novel competition, both invasive species

increased foraging efficiency, but so did *G. geiseri*. In response to novel predation, only *G. holbrooki* decreased consumption and activity and increased refuge use. No antipredator response was observed in *G. affinis*. We found consistent differences, however, between invasives and non-invasives in foraging behavior. Both in the presence and absence of the competitor and the predator, invasives foraged more efficiently and consumed more prey than non-invasives.

Keywords Behavioral plasticity · Foraging behavior · Fish · Invasion · Novelty

Introduction

Although all communities appear susceptible to invasion (Usher 1988; Williamson 1996; Lonsdale 1999), the invasibility of communities is known to vary and be affected by factors such as trophic structure and species interactions (Mack et al. 2000; Sakai et al. 2001). In particular, biotic interactions (i.e., competition and predation) may act to enhance the “resistance” of recipient communities to invasion (Elton 1958; Moyle and Light 1996). A species’ ability to invade is strongly affected by how arriving organisms respond to these biotic interactions (Shea and Chesson 2002). Predation often results in the prevention of establishment and the deceleration of spread (Lodge 1993; Jaksic 1998; Reusch 1998). Competition with native species can lower resources available to the invading species and may contribute to invasion failure (Tilman 1997; Case and Crawley 2000). Consequently, communities with higher diversity (i.e., with potentially more intense competition and/or more predators) are generally found to be more resistant to invasion (Knops et al. 1999; Stachowicz et al. 1999; Tilman 1999; Kennedy et al. 2002; but see Stohlgren et al. 1999). Conversely, communities where human activity has disrupted species assemblages and community structure (i.e., those with empty niches) often appear more prone to

Communicated by P. Bednekoff

J. S. Rehage (✉) · B. K. Barnett
Biological Sciences,
University of Kentucky,
Lexington, KY 40506, USA
e-mail: rehagej@fui.edu
Tel.: +1-305-3487314
Fax: +1-305-3481986

J. S. Rehage · A. Sih
Environmental Science and Policy,
University of California,
Davis, CA 95616, USA

Present address:

J. S. Rehage, Department of Biological Sciences,
Florida International University,
University Park, Miami, FL 33199, USA

invasion (Moyle and Light 1996; Vitousek et al. 1996; Williamson 1996).

While many studies address how species interactions affect the invasibility of communities, little research has explored how a species' ability to cope with these interactions affects invasiveness. The response (or lack thereof) of a non-indigenous species to novel predators and competitors in the new community will likely affect its overall invasion success and potential for impact. Invading organisms may escape coevolved enemies when arriving to a new community (Mitchell and Power 2003; Torchin et al. 2003), but will likely gain a suite of novel enemies to which they lack adaptive responses (Maron and Vila 2001). If novel enemies are somewhat similar to natural enemies, organisms may respond adaptively because they are essentially "preadapted" to new conditions (Sakai et al. 2001). If enemies in the new community are truly novel, phenotypic plasticity may allow organisms to respond adaptively (Vermeij 1996; Hänfling and Kollman 2002). In particular, behavioral plasticity may help organisms to respond appropriately to novelty (Schlaepfer et al. 2002) and invade (Sol and Lefebvre 2000). For instance, in birds, the best-studied animal invasive taxon (Kolar and Lodge 2001), behavioral flexibility is correlated with invasion success. Bird species with a higher frequency of foraging innovations are more likely to invade when introduced (Sol and Lefebvre 2000; Sol et al. 2002). Alternatively, invasive species may cope well with novel competitors and predators, not because they exhibit plastic responses to novel interactions per se, but simply because they are generally superior competitors (e.g., better foragers; Petren and Case 1996) and/or highly responsive to predation risk (e.g., higher response to alarm cues; Hazlett et al. 2003).

In the present study, we used laboratory assays of behavioral responses to novel competition and predation to test the idea that the ability to cope well with novel biotic challenges is a component of invasiveness. Short-term behavioral assays are a useful tool in ecology, often yielding important insights into species interactions (e.g., Milinski and Heller 1978; Gilliam and Fraser 1987; Abrahams and Dill 1989). Short-term assays are also helpful in generating predictions about longer-term population-level responses to novel species interactions (Rehage 2003). Specifically, we quantified the foraging and behavioral responses of two invasive mosquitofish species (*Gambusia affinis* and *G. holbrooki*) to novel predators and competitors, and compared their responses to those exhibited by two closely related species of lower invasion potential, *G. geiseri* and *G. hispaniolae*. Small-mouth bass (*Micropterus dolomieu*) were used as novel predators and fathead minnows (*Pimephales promelas*) as novel competitors.

We compared the foraging success, foraging efficiency, activity, and refuge use of the four *Gambusia* species in the presence and absence of these novel predators and competitors. We expected *G. affinis* and *G. holbrooki* to be more likely to respond appropriately to novelty than their less invasive relatives. Specifically, in response to

novel competition, we expected invasive *Gambusia* to increase foraging activity and efficiency in order to achieve at least the same foraging success as in the absence of fathead minnows. Aggression should be an important component of interference competition, and since *G. affinis* and *G. holbrooki* are notorious for their aggressive behavior (Courtenay and Meffe 1989), we quantified agonistic interactions between *Gambusia* and fathead minnows, with the expectation that the two invasive species would be more aggressive than *G. geiseri* and *G. hispaniolae*.

In response to novel predation risk, we expected invasive *Gambusia* to decrease foraging and activity, and increase refuge use. We quantified predator inspections with the expectation that if invasive *Gambusia* were more likely to respond adaptively to novel predation risk, they would inspect predators more. Finally, we measured predator activity to test the notion that if invasive and non-invasive *Gambusia* differ in antipredator behavior and vulnerability, then predators might respond differently to them (e.g., Quinn and Cresswell 2004). Beyond testing for plasticity in behavioral responses to novel interactions, we also considered species differences in baseline behavior to test whether *G. affinis* and *G. holbrooki* might exhibit traits such as higher overall foraging success, foraging efficiency, and activity relative to *G. geiseri* and *G. hispaniolae*.

System

The comparison of species with common ancestry such as congeners is a useful tool for identifying traits associated with invasiveness (Mack 1996). This approach minimizes the confounding effects of phylogeny (Harvey and Pagel 1991) and may clarify the importance of small differences in the ecology of species in the context of invasions (Williamson 1996). Ideally, a comparative study aimed at understanding the relationship between traits and invasiveness would map all potentially important traits on a phylogeny that includes numerous species (and numerous populations) that represent multiple, evolutionarily independent transitions between invasive and non-invasive states. However, logistical constraints limit both the number of species and the number of traits that may be examined. In this study, the four species examined represent a non-random sample of all *Gambusia*. In this poeciliid genus, it appears that only two species are invasive (*G. affinis* and *G. holbrooki*). Despite being sister taxa, research shows that they differ in traits that may affect their relative invasiveness; thus, both invasive species were included in this study. Scribner (1993) reported that *G. holbrooki* mature at a younger age and larger size than *G. affinis*, and in experimental mesocosms, *G. holbrooki* had higher population sizes, carrying capacities, and overwintering survival. In contrast, our previous work found no differences in individual growth, maximum feeding rates, population sizes, or overwintering survival, but detected higher fecundity in *G. holbrooki* and higher

dispersal tendency in *G. affinis* (Rehage 2003; Rehage and Sih 2004; Rehage et al., unpublished data). Among less invasive *Gambusia*, *G. geiseri* is the closest relative to the invasive species pair (partial phylogeny by Lydeard et al. 1995), and hence an obvious choice for comparison. Finally, since so many species in this genus have a Caribbean distribution, we included a representative of those species—*G. hispaniolae*.

Because of their ability to spread widely and their negative impacts on aquatic communities, *G. affinis* and *G. holbrooki* have been designated among the 100 worst invasive species worldwide (ISSG 2000). Efforts to reduce the incidence of malaria resulted in the widespread introduction of mosquitofish to over 70 countries as biocontrol agents against mosquitoes (Krumholz 1948; Lever 1996; Hoddle 2004). While mosquitofish have often been released in highly disturbed or artificial habitats, they eventually spread into pristine areas (Arthington and Lloyd 1989) where they severely impact native fishes, amphibians, and invertebrates (Schoenherr 1981; Courtenay and Meffe 1989; Gamradt and Kats 1996; Howe et al. 1997; Webb and Joss 1997; Goodsell and Kats 1999). Their impact is primarily through predation, usually of the eggs, fry, and larvae of native biota (Meffe 1985; Courtenay and Meffe 1989; Gamradt and Kats 1996); thus our focus on how novel interactions may affect their foraging success and underlying behaviors.

The congeners *G. geiseri* and *G. hispaniolae* were selected for comparison because of their seemingly low invasive potential. *G. geiseri* is native to spring-fed headwaters of the southwestern United States (Hubbs and Springer 1957). While several introductions have been conducted in similar habitats (Fuller et al. 1999), *G. geiseri* has failed to invade beyond points of introduction (C. Hubbs, personal communication). *G. hispaniolae* is native to the southwest region of the Dominican Republic (Burgess and Franz 1989) and has never been introduced outside its native range. However, the fact that *G. hispaniolae* has failed to spread despite increased connectivity in its native range (due to irrigation canals), while other species have (C. Rodriguez, personal communication), may suggest that *G. hispaniolae* is non-invasive.

Methods

To examine how invasive and non-invasive *Gambusia* species respond to novelty, we exposed wild *Gambusia* females to a novel predator and a novel competitor in two separate, consecutive laboratory experiments. Novel predators and competitors were chosen from species that had no evolutionary history (i.e., no coexistence) with the four *Gambusia* study populations and from widespread invasive species that spreading *Gambusia* would likely encounter. Because of our interest in exploring correlations in feeding performance and behavioral responses across predation and competition contexts (reported elsewhere), we used the same individual females in both experiments. Females were collected from within each species' native ranges in the summer and fall of 1999. *G. geiseri* and *G. affinis* females were collected from Comal springs and the Comal river (1.5 km apart), respectively in Comal County, TX. *G. holbrooki* females came from Leon Hines Lake, Escambia

County, AL., and *G. hispaniolae* females were collected from La Azufrada spring, Lake Enriquillo, Dominican Republic. We chose adult females because they are the gender and age class that is most relevant for initial invasions. *Gambusia* females can store sperm from multiple males; thus a single female can initiate a population with minimal negative founder effects (Chesser et al. 1984; Zane et al. 1999).

In order to document the effect of novel behavioral interactions on the foraging behavior of *Gambusia*, we conducted behavioral trials in the presence of prey. We used *Daphnia pulex* as prey in the competition experiment and *Drosophila melanogaster* as prey in the predation experiment. Stomach-content analyses show that cladocerans (including daphniids) and neustonic prey often constitute a significant proportion of the diet of invasive *Gambusia* (Crivelli and Boy 1987; Arthington and Marshall 1999; Garcia-Berthou 1999). No data were found on the diet of natural populations of *G. geiseri* and *G. hispaniolae*. All study fish were previously fed both prey items (Rehage et al., unpublished data); thus prey were not novel. We used the same total prey biomass in competition and predation trials (0.02 g) but different prey densities (40 *Daphnia* vs 20 *Drosophila*).

Novel competition

Fathead minnows were chosen as novel competitors because they exhibit significant overlap in resource use with *Gambusia* (i.e., they are potential competitors), but do not co-occur with focal *Gambusia* populations. *Gambusia* species are likely to encounter minnow-like competitors in both their native range and introduced ranges (Hubbs et al. 1953; Arthington 1991). Fathead minnows are very similar to invasive mosquitofish in their habitat requirements (usually shallow and vegetated areas in lakes and streams), broad diets, and wide physiological tolerances (Moyle 2002). Fathead minnows are themselves widespread invaders, introduced as a bait and forage fish (Fuller et al. 1999). Minnows used in the experiment were obtained from the Frankfort Fish Hatchery, Frankfort, KY.

We performed two 10-min trials on 12 females of each *Gambusia* species (12 females \times 4 species \times 2 trials = 96 trials). Trials were conducted in April 2000 and videotaped for later analysis. Trials without the competitor (referred to as individual trials) were conducted first (8–13 April). *Gambusia* were randomly assigned to one of the six consecutive trial days. Trials with minnows present (competition trials) were conducted later over a 5-day period (19–24 April). The order of trials was the same in competition trials as in individual trials. All trials were conducted in 6-l plastic containers where individual *Gambusia* had been housed for several months prior to the experiment. Two days prior to the start of the experiment, 48 randomly chosen but size-matched (within 1 mm standard length) fathead minnows were isolated into identical tanks. Because *Gambusia* maximum size is typically around 45 mm and fathead minnow maximum size is closer to 100 mm (Fuller et al. 1999), all minnows used in the experiment were juveniles.

Individual trials were started immediately upon addition of the 40 *Daphnia pulex* and ended when all prey were consumed or after 10 min. In the competition trials, the minnow was added to the *Gambusia* tank and allowed to acclimate for 24 h prior to trials. A translucent, perforated partition placed in the center of each tank allowed chemical and visual cues to be transmitted but no physical contact between the pair. The partition was removed 5 min before the start of each competition trial. Trials started with the addition of 40 *Daphnia pulex* (same number of prey as in individual trials). All fish were starved 24 h prior to trials. In between trials, fish were fed commercial fish flakes ad libitum, and tanks were provided with refuge in the form of a clump of java moss, *Vesicularia dubyana*. Water temperature and photoperiod were maintained at about 22.3°C (± 0.1 SE, $n=96$) and 14 h L:10 h D for the duration of the experiment.

From observations conducted during trials, we recorded the number of prey consumed by each competitor (foraging success). For the competition trials, we calculated the proportion of the prey

consumed by the *Gambusia* out of the total consumption [prey eaten by *Gambusia*/(prey eaten by *Gambusia*+prey eaten by minnow)]. From analyses of taped trials, we extracted measures of *Gambusia* foraging efficiency and activity with and without competition, and when minnows were present, we obtained data on aggression. Taped trials (top-view) were watched 2–3 times by a single observer, using a stopwatch to measure time spent in each behavior and a counter to record the number of agonistic interactions. Foraging efficiency was calculated as the number of prey consumed per unit time spent foraging. Foraging time was scored as the time spent encountering, attacking, capturing, and consuming (handling) prey, but it did not include prey search time (difficult to assess in the confinement of small tanks). Because time spent foraging could vary substantially across trials, patterns of foraging efficiency did not necessarily mirror patterns of foraging success.

Foraging time included both successful and unsuccessful encounters, attacks, and captures. An encounter was defined as any approach to a prey item, even if there was no attempt to capture it. Encounters were clearly distinguished from non-foraging swimming because, in an encounter, *Gambusia* swam directly towards a prey item, approaching it closely, and often bumping it. An attack was defined as any attempt to seize a prey item, and a capture was a successful attack. Time spent handling prey was defined as the time between capture and consumption (i.e., when the prey item was actually swallowed). Consumption was indicated by the cessation of movement of mouthparts and the resumption of swimming. *Gambusia* typically ceased or slowed their swimming to handle prey (J.S. Rehage, personal observation).

Inactivity was recorded as the proportion of time *Gambusia* spent completely motionless (time spent motionless divided by trial duration). We were careful not to score the cessation of movement while handling prey as inactivity. For interspecific aggression, we calculated a rate of agonistic interactions (number per minute) by dividing the number of interactions initiated by the *Gambusia* or the minnow separately by the trial duration. These interactions included approaches, chases, and chases with physical contact (usually a bump or bite in the abdominal area). A rapid, direct movement by one fish toward another fish within less than one body length (about 35 mm) was considered an approach. If the fish being approached swam away from the fish doing the approaching and the latter followed, this was considered a chase.

Novel predation

Smallmouth bass were selected as the novel predator species because they are widespread and abundant, and like fathead minnows, do not co-occur with study populations. Smallmouth bass occur naturally in the upper Mississippi River drainage, but like other predatory centrarchids, they have been widely introduced and are presently a common predator in many North American lakes and streams (Fuller et al. 1999). Because other centrarchid species (e.g., largemouth bass and *Lepomis* sp.) coexist with and may predate upon three of the four *Gambusia* species (*G. affinis*, *G. holbrooki* and *G. geiseri*, but not *G. hispaniolae*—cichlids are their natural predators), the degree to which smallmouth bass represent a completely novel threat may vary. However, even closely related centrarchids, such as smallmouth and largemouth bass, differ in their predatory behavior (Winemiller and Taylor 1987; Hodgson et al. 1997), making smallmouth a relatively novel threat to all *Gambusia*. For this experiment, four juvenile smallmouth bass (averaging 130 mm standard length) were collected from the confluence of the north and south forks of Elkhorn Creek, Franklin County, KY.

As in the competition experiment, trials lasted 10 min and were videotaped for later analysis. The 12 females of each *Gambusia* species were subjected individually to a no-predator trial (also referred to as the individual trial) followed by a trial with a smallmouth bass present (predation trial) (12 females×4 species×2 trials=96 trials). Trials were conducted in September 2000 in six 3-day blocks. Two fish of each *Gambusia* species were randomly assigned to each time block. On day 1 of each block, female *Gambusia* were introduced to 38-l aquaria (after being fed flakes ad

libitum) and were starved for 24 h. On day 2, individual trials were conducted with 20 flightless, live fruit flies (*Drosophila melanogaster*) added to the water surface. Prey left unconsumed at the end of individual trials were counted and left in the tank for an additional 15 min to allow fish to continue feeding, in order to better standardize hunger levels prior to predation trials. *Gambusia* were then starved for the next 23.5 h. On day 3, predation trials were conducted with the same number of flies, but in the presence of the smallmouth bass. Trials were randomized so that each of four individual predators encountered three females of each *Gambusia* species.

One hour prior to the predation trials, we divided tanks into equal halves using an opaque, plastic partition, and placed the bass in the side opposite to the *Gambusia*. Partitions were later removed, and after a 5-min acclimation period, *Drosophila* were added and trials were started. Tanks were provided with a refuge for the *Gambusia* consisting of a piece of PVC tubing (100 mm length by 50 mm diameter) glued to the side of the tank 10 mm below the water line. Refuges were placed high in the water column because *Gambusia* are typically found close to the surface, and under predation risk often move to shallow areas where predators are excluded. Predators were allowed to move freely in tanks and approach *Gambusia*. Predators were fed *Gambusia* ad libitum in between trials but were starved 24 h prior to trials. After the first 3-day block, three of the four predators died (due to low oxygen conditions in holding tanks) and were replaced with fish from the same population. There were 3 actual predation events during the experiment, but we were able to replace only 1 female (final sample size was 46 *Gambusia*).

Trials were terminated either when all flies were consumed or after 10 min. From direct observations during trials, we quantified the number of prey consumed in the presence and absence of predators (foraging success). From tapes, we quantified *Gambusia* foraging efficiency, activity, and refuge use in the absence and presence of smallmouth bass, and predator inspections when bass were present. Foraging efficiency and activity were scored from tapes in the same manner as in the competition trials. Refuge use was the proportion of the trial time spent in refuge. We considered *Gambusia* to be in refuge if fish were found either on top, inside, behind (between refuge and back wall of tank) or right underneath the PVC tubing refuge. Fish scored to be in refuge could be swimming or inactive, so that inactivity and refuge use were not mutually exclusive behaviors. Predator inspections were cautious approaches by the *Gambusia* toward the predator and were scored as a rate (number of approaches divided by trial duration). Inspections were distinguished from normal swimming because inspecting *Gambusia* slowly approached the predator while being visually fixated on it, and when done inspecting, generally retreated from the predator without losing eye contact.

Since we expected that predator activity would affect the perception of risk by female *Gambusia*, and thus their behavioral responses, and since individual predators differed in how active they were during trials, we quantified the proportion of time predators were active in each trial. Predators were considered to be active if there was any movement of their body, including movement of their fins that involved only a slight change in position, as well as regular swimming.

Statistical analyses

For both experiments, we examined species differences in behavioral and foraging responses to novel competitors and predators by running repeated measures ANOVAs, with species as the between-subject effect and competition (or predation) and the competition (or predation) by species interaction as the within-subject effects. The species by competition (or predation) effect was indicative of differences in behavioral plasticity, whereas the species effect indicated overall behavior differences. Focal variables included: foraging success, foraging efficiency, proportion of time inactive, and proportion of time in refuge (only measured in predation trials). Prior to running ANOVAs, a MANOVA was per-

formed to test for an overall species effect on mean response variables (average of with and without competition and with and without predation trait values). Preliminary analyses included predator activity and *Gambusia* size as covariates and time as a blocking factor (trial day in competition experiment and 3-day block in predation experiment); however, these factors were not significant and were excluded from final analyses. The only exception was *Gambusia* size being a significant covariate in the analysis of competition foraging success and foraging efficiency. However, the only effect of size was to increase the disparity between the two non-invasive species (since they are at the opposite ends of the size distribution—*G. hispaniolae* was the largest, *G. geiseri* the smallest, and the invasive pair were intermediate in size). Since including size complicated interpretation of results otherwise, we omitted it from analyses reported here.

Simple one-way ANOVAs for the competitor and predator treatment only were conducted with species as a main effect (neither covariate was significant). Separate analyses were conducted for the following variables: proportion of prey eaten by *Gambusia* in competition, rates of predator inspections by *Gambusia*, and predator activity. Aggressive interactions in competition trials (calculated as rates, number per minute of trial time) were compared with a repeated measures ANOVA that tested for the effect of competitor (*Gambusia* vs minnow), of *Gambusia* species, and the interaction.

To meet parametric test assumptions, we performed Cochran's test (Underwood 1997) on all variables, and transformed all those where evidence of variance heterogeneity was found. Among the competition variables, proportion of time inactive, proportion of prey consumed by *Gambusia*, and aggression rates were transformed; whereas for predation variables, foraging efficiency, proportion of time in refuge, and predator inspection rates were transformed. Transformations were log (ln of observed value+1) for rates and arcsine square root for proportions.

When a significant species effect was found, we used planned orthogonal contrasts to test three hypotheses of interest. We tested whether the two invasive species differed from the two non-invasive relatives and whether there were differences between the two invasive species (*G. affinis* vs *G. holbrooki*) and between the two non-invasive species (*G. geiseri* vs *G. hispaniolae*). For significant species by competition (and predation) interactions in the repeated measures, we compared means in the absence and presence of biotic interactions for each species separately (four comparisons). We used Bonferroni pairwise comparisons by adjusting the significance level to $P=0.0125$ (0.05/4) (Miller 1981). All statistical tests were conducted using the mixed model procedure in SAS 9.1 (Littell et al. 1996).

Results

Our MANOVA analysis of behavioral and foraging variables averaged across competition/no competition and predation/no predation contexts showed a significant species effect (Wilks's Lambda, $F_{21,75}=2.5$, $P=0.002$), indicating that there was variation in how the four *Gambusia* species foraged and generally behaved in both experiments.

Novel competition

Foraging success

Gambusia species differed in their overall foraging success, but showed no differential response to novel competition (no significant species×competition interaction)

Table 1 Summary of repeated measures and one-way ANOVAs testing foraging variables and behavioral responses in the competition experiment

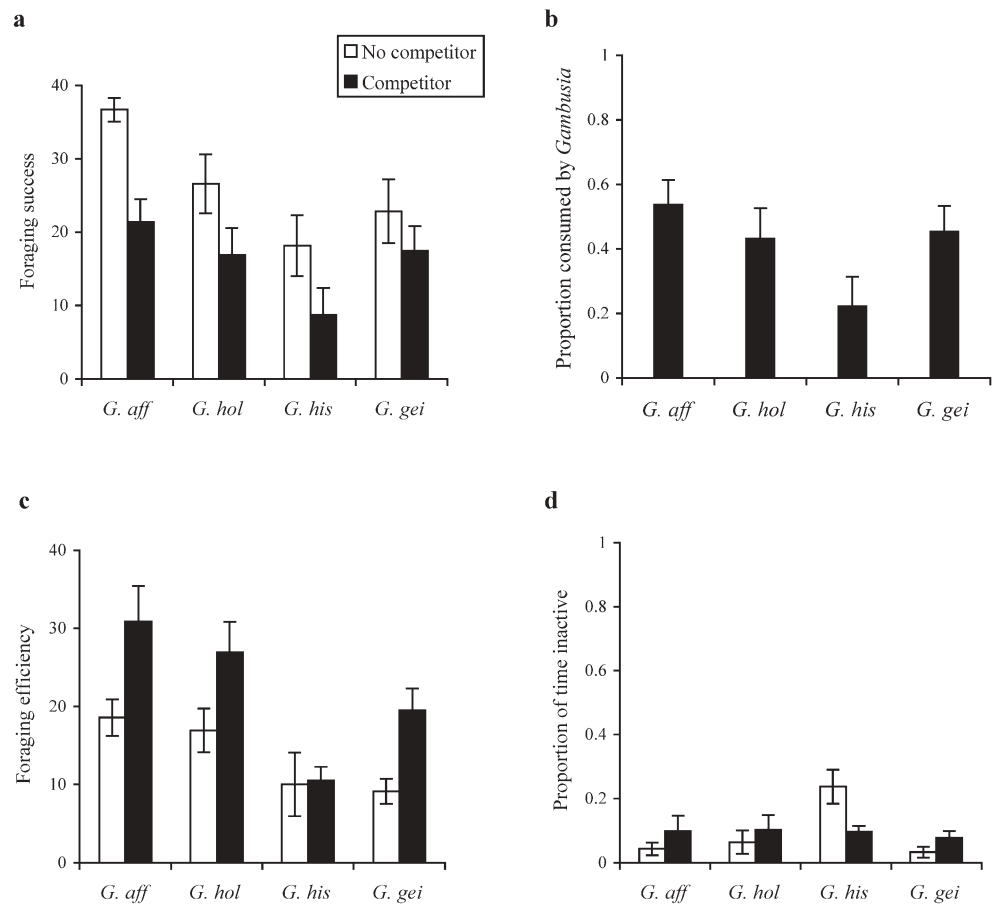
Variable	Effect	df	F	P-value
Foraging success	Species	3, 44	4.6	0.007
	Competition	1, 44	25.3	0.0001
	Species×competition	3, 44	1.1	0.37
Foraging efficiency	Species	3, 44	6.3	0.001
	Competition	1, 44	17.1	0.0001
	Species×competition	3, 44	2.4	0.08
Inactivity	Species	3, 44	5.1	0.004
	Competition	1, 44	0.03	0.86
	Species×competition	3, 44	8.7	0.0001
Agonistic interactions	Species	3, 44	1.4	0.27
	Competitor identity	1, 44	12.8	0.001
	Species×competitor identity	3, 44	1.2	0.34
Proportion of prey consumed by <i>Gambusia</i>	Species	3, 44	3.0	0.04

(Table 1). Regardless of whether or not competitors were present, prey consumption was higher for the invasive *G. holbrooki* and *G. affinis* relative to *G. hispaniolae* and *G. geiseri* (25 vs 17 *Daphnia*) ($F_{1,44}=8.4$, $P=0.006$). Of the two invasives, *G. affinis* tended to be the species with the greatest overall consumption (compared to *G. holbrooki*; $F_{1,44}=3.0$, $P=0.09$) (Fig. 1a). The overall effect of competition was a 40% decrease in consumption across all species, but in competition trials we saw differences in the number of prey consumed by *Gambusia* relative to the total consumption (Table 1). *G. geiseri* and *G. hispaniolae* secured a lower proportion of the total prey consumed relative to the invasive species pair ($F_{1,44}=3.4$, $P=0.04$). In particular, while invasive *Gambusia* and *G. geiseri* secured about half of the prey eaten (47% compared to 53% eaten by minnows), *G. hispaniolae* only consumed 22% of the prey (compared to *G. geiseri*, $F_{1,44}=5.0$, $P=0.03$) (Fig. 1b).

Foraging efficiency

The repeated measures analysis of foraging efficiencies detected a competition effect, a species effect, and a trend for a competition by species interaction (Table 1). Across competition and no competition contexts, invasive *Gambusia* were significantly more efficient foragers than the two non-invasive species ($F_{1,44}=16.5$, $P=0.0002$). On average, invasive *Gambusia* consumed 23 prey per minute spent foraging, whereas non-invasive *Gambusia* consumed just 12 prey per minute. Interestingly, competition caused an overall increase in *Gambusia* foraging efficiency (i.e., all fish generally foraged more intensely when a competitor was present; Fig. 1c), but we also detected a tendency for species to respond differently. Foraging efficiencies appeared to increase for *G. affinis* and *G. geiseri*, and—though not significantly—for *G. holbrooki* (Bonferroni pairwise comparisons with critical

Fig. 1 a Foraging success (number of *Daphnia pulex* consumed). **b** Proportion of *Daphnia* consumed while in competition. **c** Foraging efficiency (*Daphnia* consumed per minute spent foraging). **d** Proportion of trial time spent inactive in the presence and absence of a novel competitor (by *Pimephales promelas*) of each *Gambusia* species, the invasives *G. holbrooki* and *G. affinis*, and the non-invasives *G. geiseri* and *G. hispaniolae*. Data are means \pm 1 SE.



$P=0.0125$; $P=0.001$, $P=0.006$, and $P=0.04$ respectively). In contrast, *G. hispaniolae*, the non-invasive species that ate only 22% of the *Daphnia* in the presence of minnows, showed no increase in foraging efficiency ($P=0.89$).

Activity

Gambusia were very active in the competition experiment (Fig. 1d). On average, females spent only 10% of trial time immobile, and we did not detect a large difference between invasive and non-invasive *Gambusia* ($F_{1,44}=2.8$, $P=0.10$). Instead, the largest difference was between *G. hispaniolae* and *G. geiseri* ($F_{1,44}=12.2$, $P=0.001$). On average, *G. hispaniolae* spent more than twice as much time immobile as *G. geiseri*. In terms of a plastic response to competition, only *G. hispaniolae* changed its activity significantly between the competition/no competition contexts by cutting its time spent immobile in half ($P=0.0001$) (Fig. 1d); however, this increase in activity did not translate into better foraging performance since efficiency did not increase concurrently. We suspect this increase in activity resulted from chases by minnows.

Aggression

Contrary to expectations, we found fathead minnows to be significantly more aggressive than *Gambusia* (Table 1). The number of agonistic interactions initiated over all trials totaled 629 for minnows compared to only 282 for *Gambusia*, and interaction rates (adjusted for trial duration) were, on average, 3 times greater for minnows relative to *Gambusia* (Fig. 2). There was no species by competitor identity interaction indicating that the *Gambusia* species were equally aggressive toward minnows, and minnows were also equally aggressive toward invasive and non-invasive *Gambusia*.

Novel predation

Foraging success

In parallel with the competition experiment, invasive *Gambusia* consumed more prey than the two non-invasive species in both the presence and absence of predation risk (on average 12 vs 7 *Drosophila*) ($F_{1,42}=8.1$, $P=0.007$) (Fig. 3a). Novel predation risk from smallmouth bass resulted in a 22% decrease in consumption across *Gambusia* species (Table 2), although on a species by species basis, only invasive *G. holbrooki* reduced foraging sig-

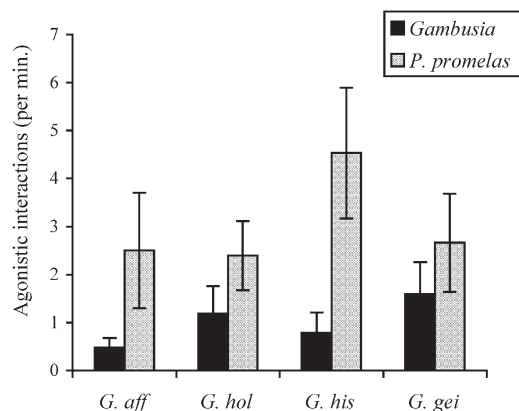


Fig. 2 Number of agonistic interactions (means \pm 1 SE) per minute of trial time initiated by each *Gambusia* species and by *P. promelas* in competition trials.

nificantly (by 46%) ($P=0.0003$), no significant response was detected in the other species (*G. affinis*, $P=0.21$; *G. geiseri*, $P=0.80$; *G. hispaniolae*, $P=0.46$).

Foraging efficiency

There was a species difference, though not significant, in overall foraging efficiency (Table 2). On average, invasives consumed 7 prey per minute spent foraging in both trials, whereas non-invasives consumed 4 prey per minute

Table 2 Summary of repeated measures and one-way ANOVAs testing foraging variables and behavioral responses in the predation experiment

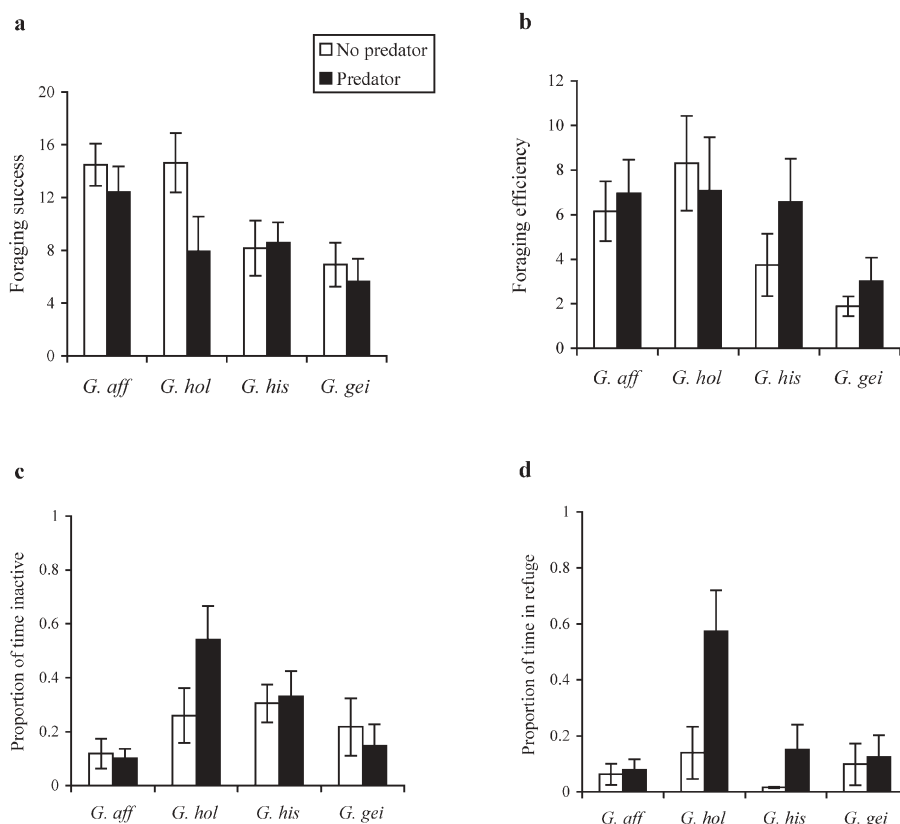
Variable	Effect	df	F	P-value
Foraging success	Species	3, 42	3.2	0.03
	Predation	1, 42	8.4	0.006
	Species \times predation	3, 42	3.3	0.03
Foraging efficiency	Species	1, 42	2.6	0.07
	Predation	3, 42	0.6	0.43
	Species \times predation	3, 42	3.2	0.04
Inactivity	Species	3, 42	3.1	0.04
	Predation	1, 42	1.6	0.23
	Species \times predation	3, 42	3.0	0.04
Refuge use	Species	3, 42	3.6	0.02
	Predation	1, 42	14.7	0.0001
	Species \times predation	3, 42	4.9	0.005
Predator inspections	Species	3, 42	0.88	0.46
Predator activity	Species	3, 42	0.25	0.86

($F_{1, 42}=4.8$, $P=0.03$). In response to predation risk, we saw a plastic response only in *G. hispaniolae*, with an increase in foraging efficiency in the presence of the predator ($P=0.011$) (Fig. 3b). No response was detected in the other species (*G. affinis*, $P=0.85$; *G. holbrooki*, $P=0.10$; *G. geiseri*, $P=0.59$).

Activity, refuge use and predator inspections

Unlike competition, predation risk had a strong effect on activity levels, and this effect was parallel to the effect

Fig. 3 a Foraging success (number of *Drosophila melanogaster* consumed). **b** Foraging efficiency. **c** Proportion of trial time spent inactive. **d** Proportion of trial time spent in refuge of each *Gambusia* species in the presence and absence of novel predation risk (by *M. dolomieu*). Data are means \pm 1 SE.



seen on refuge use. Neither activity nor refuge use varied significantly between invasive and non-invasive *Gambusia* ($F_{1,42}=0.01$, $P=0.90$ and $F_{1,42}=3.1$, $P=0.09$ respectively). The main difference in these behaviors was between *G. holbrooki* and *G. affinis* (Activity: $F_{1,42}=7.6$, $P=0.009$; and refuge use: $F_{1,42}=8.0$, $P=0.007$). In the presence of smallmouth bass, *G. holbrooki* females more than doubled their time spent immobile and quadrupled refuge use ($P=0.003$ and $P=0.0001$ respectively), whereas no response was observed in the other three species (Fig. 3c,d).

There were no species differences on the rate of predator inspections (Table 2). Overall, a fairly small number of predator inspections were recorded during predation trials (56 total). This may be explained by the fact that trials were conducted in relatively small tanks where *Gambusia* may have been able to assess risk without expressly approaching the predator. We also found no indication that predators responded differently toward invasive and non-invasive *Gambusia* by altering their activity (Table 2).

Discussion

Invaders have particularly large impacts on a recipient community if the invader performs a novel function (Elton 1958; Ricciardi and Atkinson 2004). This novelty in function has been linked to species extinctions, shifts in community structure (e.g., predators on oceanic islands and lakes; Fritts and Rodda 1998; Ogutu-Ohwayo 1999) and even changes in key ecosystem processes (e.g., nutrient cycling; Vitousek et al. 1997). However, how the invaders themselves deal with novel interactions associated with invading a new community is less well understood. Similarly, the role of behavior as a key factor mediating a species' invasion success and impact has remained largely unexplored until relatively recently (Holway and Suarez 1999; Sol et al. 2002). Our study compared behavioral responses between invasive and non-invasive congeners with the expectation that invasives may be more likely to respond appropriately to novel interactions because of greater behavioral plasticity. We documented changes in behavior in all variables measured, in both contexts (predation and competition), and in all four *Gambusia* species; however, we did not find consistent differences between invasive and non-invasive *Gambusia* in their behavioral responses to novel interactions. Both invasive *Gambusia* responded adaptively to novel competition by increasing foraging efficiency, but only *G. holbrooki* showed a change in behavior in response to novel predation risk. In the presence of smallmouth bass, *G. holbrooki* decreased foraging success and activity and increased refuge use, while *G. affinis* exhibited no response. Among the non-invasives, *G. geiseri* responded adaptively to novel competition with increased efficiency but not to predation risk, while *G. hispaniolae* only increased activity in response to competition and showed what may be considered a mal-

adaptive response to predation (increased foraging efficiency).

In contrast, our study documented consistent differences in the foraging behavior of the four *Gambusia* species. Both in the presence and absence of the novel competitor and novel predator, *G. holbrooki* and *G. affinis* foraged more efficiently and consumed more prey than *G. geiseri* and *G. hispaniolae*. In a previous experiment conducted in the absence of biotic interactions, *G. affinis* and *G. holbrooki* also exhibited higher feeding rates on the same two prey used here and on a third prey item, *Lirceus fontinalis*, than their non-invasive congeners (Rehage et al., unpublished data). These results suggest that a superior foraging ability may be an important component of the invasiveness of *G. holbrooki* and *G. affinis*. Previous studies on the foraging behavior of invaders have largely tested whether diet breadth (among other traits) is a good predictor of invasiveness (e.g., Kolar and Lodge 2002), while little attention has been given to how efficient, opportunistic or aggressive invaders are as foragers. However, a superior ability to acquire resources can strongly affect an invader's survival and fecundity, and may confer a large competitive advantage in the invaded range (Petren and Case 1996). Such an advantage can increase the probability of successful establishment and result in the displacement of native species (Petren and Case 1996; Bøhn and Amundsen 2001). Thus, these results also suggest that if introduced, *G. geiseri* and *G. hispaniolae* should have relatively lower per capita impact. In contrast, predation by mosquitofish is known to strongly impact local prey populations and can result in significant alterations in aquatic communities (Hurlbert et al. 1972; Hurlbert and Mulla 1981; Harris 1995; Margaritora et al. 2001).

Novel competition with fathead minnows affected *Gambusia* foraging success and behavior via agonistic interactions (i.e., interference competition) and through the direct consumption of resources (i.e., exploitation). Unexpectedly, fathead minnows proved to be more aggressive than invasive *Gambusia*. Minnows were more likely to chase, contact, and nip *Gambusia* than vice versa, and they significantly lowered *Gambusia* foraging success. Minnows also did not discriminate among the four *Gambusia* species; they were equally aggressive toward invasive and non-invasive species. Similarly, invasive and non-invasive *Gambusia* showed equally low levels of aggression towards minnows. These results are surprising given that several studies make reference to the high aggression of mosquitofish as an important mechanism explaining their severe impact in invaded communities (Myers 1965; Schoenherr 1981; Meffe 1985; Courtenay and Meffe 1989). Aside from their predatory impact, the agonistic behavior of mosquitofish is known to cause harmful physiological stress on native species (e.g., cessation of feeding, decreased fecundity, and increased mortality), even on species much larger than themselves (Meffe 1985; Courtenay and Meffe 1989; Howe et al. 1997). In other systems, recent studies also show that aggression is an important component of both

an invader's invasiveness and impact (Usio et al. 2001; Tsutsui et al. 2003).

Despite being more aggressive than *Gambusia*, fathead minnows did not generally monopolize prey resources. Invasive *Gambusia* species faced with competition had lower foraging success (relative to when competitors were absent), but competition for prey between invasives and fathead minnows was highly symmetrical. An increase in foraging efficiency allowed the invasive species to cope with competition rather well. Surprisingly, non-invasive *G. geiseri* showed the same adaptive response to novel competition. Only *G. hispaniolae* showed no response to competition (beyond an increase in activity), thus securing a smaller portion of the prey. This lack of response may be explained by the fact that *G. hispaniolae* females inhabit a species-poor community with only two known potential competitors, *Limia melanotata* (another poeciliid species) and *Cyprinodon bondi* (Haney and Walsh 2003), whereas other *Gambusia* likely interact with a larger number of potential competitors (Hubbs et al. 1953; USFWS 1996).

In response to predation risk, prey typically decrease their activity and foraging behavior, and alter their habitat use (i.e., increasing refuge use or use of predator-free microhabitats) (Sih 1987; Lima and Dill 1990). These responses are adaptive since they often reduce conspicuousness, encounter rates, and thus vulnerability to predators (i.e., Lawler 1989; McPeck 1990). This is especially true in the case of visual ambush predators like smallmouth bass that respond to prey movement (Edmunds 1974). In our predation experiment, *G. holbrooki* responded strongly to the presence of smallmouth bass by reducing food consumption and activity and increasing refuge use. Predation risk had little or no effect on the foraging success or behavior of the other three *Gambusia* species, including invasive *G. affinis*. The only detectable response was an increase in foraging efficiency in *G. hispaniolae*. This response may be considered inappropriate since increased foraging may make prey more conspicuous to predators, and may reduce vigilance and escape success. This risky behavior could be explained by a higher energetic stress in *G. hispaniolae* relative to the other *Gambusia* (Godin and Smith 1988). Alternatively, an increase in foraging efficiency might actually be adaptive if it results in less time exposed to predation risk.

Since invading species are likely to encounter both novel predators and competitors as they colonize new habitats, invasion success could depend on the ability of invading organisms to respond adaptively to both (as well as other novel enemies; e.g., pathogens, parasites). Only *G. holbrooki* responded adaptively to both competition and predation, suggesting that *G. holbrooki* may inherently have greater behavioral plasticity than other *Gambusia*. Alternatively, these differences may be due to the fact that the *Gambusia* species differed in their perception of the relative costs and benefits of the particular species interactions tested here. For instance, *G. holbrooki* may be more susceptible to predation by smallmouth bass than other *Gambusia* and, therefore, showed a stronger

antipredator response (e.g., Sih 1982). It is also plausible that the particular competition and predation regime experienced by *G. holbrooki* resembled native conditions enough to allow females to respond adaptively in both contexts. If this is true, differences in behavioral response simply reflect differences in learning and experience, since we used wild females. This argument may be strengthened by the fact that the two species that could experience the most similar competition and predation regimes in the wild, *G. affinis* and *G. geiseri*, although not equally invasive, responded similarly. Both species increased foraging efficiency in response to competition, and showed no response to predation risk. *G. affinis* females were collected 1.5 km downstream from the spring-fed headwaters inhabited by the *G. geiseri* females. However, despite this small distance, abiotic conditions in the springhead (i.e., constant temperature and high flow) make it an area of endemism with a unique species assemblage (USFWS 1996). Water control structures between the spring and river may further limit the degree of biotic similarity between these habitats.

While some studies point to the loss of natural enemies as a major mechanism for invasion success, other research shows that invaders often quickly gain a large number of novel enemies in a newly invaded range (Maron and Vila 2001; Colautti et al. 2004). Because invaders are essentially naïve to them, their population performance may be strongly affected by their interactions with these novel enemies (e.g., Case and Crawley 2000). In the absence of other adaptive responses, the notion that behavior should play an important role in mediating novel interactions deserves further study. In our system, further testing with additional novel predators and competitors is needed to clarify if these responses to novelty are an important component of mosquitofish invasiveness, particularly for *G. holbrooki*. To remove the potential confounding effect of experience and learning, future work should use laboratory-reared individuals. Future studies should also compare *Gambusia* behavioral responses to not just novel invasive predators and competitors, but also to native predators and competitors, and to novel predators and competitors that are not invasive themselves. These comparisons should provide a more definitive test of the hypothesis that *Gambusia* species respond differently to not just competition or predation per se, but to novel interactions, in particular.

Acknowledgements We thank C. Hubbs and C. Rodriguez for their invaluable assistance with field collections. Discussions with members of the Sih and Crowley laboratories on the role of novelty in an invasion context helped shape this research. This work was supported by an NSF graduate research fellowship and an ASIH Raney fund award to J.S.R., NSF DDIG DEB-0206542 to J.S.R., A.S. and P. Crowley, and NSF IBN 0222063 to A.S. This study complies with all current, relevant laws and animal care regulations of the United States of America.

References

- Abrahams MV, Dill LM (1989) A determination of the energetic equivalence of the risk of predation. *Ecology* 70:999–1007
- Arthington AH (1991) Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia. *Can J Fish Aquat Sci* 48:33–43
- Arthington AH, Lloyd LN (1989) Introduced poeciliids in Australia and New Zealand. In: Meffe GK, Snelson FF (eds) *Ecology and evolution of livebearing fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp 333–348
- Arthington AH, Marshall CJ (1999) Diet of exotic mosquitofish, *Gambusia holbrooki*, in an Australian Lake and potential for competition with indigenous fish species. *Asian Fish Sci* 12:1–16
- Bøhn T, Amundsen P-A (2001) The competitive edge of an invading species. *Ecology* 82:2150–2163
- Burgess GH, Franz R (1989) Zoogeography of the Antillean freshwater fish fauna. In: Woods CA (ed) *Biogeography of the West Indies*. Sandhill Crane, Gainesville, FL, pp 263–304
- Case CM, Crawley MJ (2000) Effect of interspecific competition and herbivory on the recruitment of an invasive alien plant: *Conyza sumatrensis*. *Biol Invasions* 2:103–110
- Chesser RK, Smith MW, Smith MH (1984) Biochemical genetics of mosquitofish. III. Incidence and significance of multiple insemination. *Genetica* 64:77–81
- Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success predicted by the enemy release hypothesis? *Ecol Lett* 7:721–733
- Courtenay WR, Meffe GK (1989) Small fishes in strange places: a review of introduced Poeciliids. In: Meffe GK, Snelson FF (eds) *Ecology and evolution of livebearing fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp 319–331
- Crivelli AJ, Boy V (1987) The diet of the mosquitofish *Gambusia affinis* (Baird and Girard) (Poeciliidae) in Mediterranean France. *Rev Ecol* 42:421–435
- Edmunds M (1974) *Defense in animals: a survey of antipredator defenses*. Longman, London
- Elton CS (1958) *The ecology of invasions by animals and plants*. Methuen, London
- Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annu Rev Ecol Syst* 29:113–140
- Fuller PL, Nico LG, Williams JD (1999) Nonindigenous fishes introduced into inland waters of the United States. American Fisheries Society, Special Publication 27, Bethesda, MD
- Gamradt SC, Kats LB (1996) Effect of introduced crayfish and mosquitofish on California newts. *Conserv Biol* 10:1155–1162
- Garcia-Berthou E (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *J Fish Biol* 55:135–147
- Gilliam JF, Fraser DF (1987) Habitat selection under predation hazard: test of a model with foraging minnows. *Ecology* 68:1856–1862
- Godin J-GJ, Smith SA (1988) A fitness cost of foraging in the guppy. *Nature* 333:69–71
- Goodsell JA, Kats LB (1999) Effect of introduced mosquitofish on pacific treefrogs and the role of alternative prey. *Conserv Biol* 13:921–924
- Haney DC, Walsh SJ (2003) Influence of salinity and temperature on the physiology of *Limia melanotata* (Cyprinodontiformes: Poeciliidae): a search for abiotic factors limiting insular distribution in Hispaniola. *Caribb J Sci* 39:327–337
- Hänfling B, Kollman J (2002) An evolutionary perspective of biological invasions. *Trends Ecol Evol* 17:545–546
- Harris PM (1995) Are autecologically similar species also functionally similar? A test in pond communities. *Ecology* 76:544–552
- Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford Press, Oxford
- Hazlett BA, Burba A, Gherardi F, Acquistapace P (2003) Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biol Invasions* 5:223–228
- Hoddle MS (2004) Restoring balance: using exotic species to control invasive exotic species. *Conserv Biol* 18:38–49
- Hodgson JR, He X, Schindler DE, Kitchell JF (1997) Diet overlap in a piscivore community. *Ecol Freshwater Fish* 6:144–149
- Holway DA, Suarez AV (1999) Animal behavior: an essential component of invasion biology. *Trends Ecol Evol* 14:328–330
- Howe E, Howe C, Lim R, Burchett M (1997) Impact of the introduced poeciliid *Gambusia holbrooki* (Girard 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner 1865) in Australia. *Mar Freshwater Resource* 48:425–434
- Hubbs C, Springer VG (1957) A revision of the *Gambusia nobilis* species group, with descriptions of three new species, and notes on their variation, ecology, and evolution. *Tex J Sci* 9:279–327
- Hubbs C, Kuehne RA, Ball JC (1953) The fishes of the upper Guadalupe River, Texas. *Tex J Sci* 5:216–244
- Hurlbert SH, Mulla MS (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* 83:125–151
- Hurlbert SH, Zedler J, Fairbanks D (1972) Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science* 175:639–641
- ISSG (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group, University of Auckland, Auckland
- Jaksic FM (1998) Vertebrate invaders and their ecological impacts in Chile. *Biodiversity Conserv* 7:1427–1445
- Kennedy TA, Naeem S, Howe KM, Knops JMH, Tilman D, Reich P (2002) Biodiversity as a barrier to ecological invasion. *Nature* 417:636–638
- Knops JMH, Tilman D, Haddad NM, Naeem S, Mitchell CE, Haarstad J, Ritchie ME, Howe KM, Reich PB, Siemann E, Groth J (1999) Effects of plant species richness on invasion dynamics, disease outbreaks and insect abundance and diversity. *Ecol Lett* 2:286–293
- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends Ecol Evol* 16:199–204
- Kolar CS, Lodge DM (2002) Ecological predictions and risk assessment for alien species. *Science* 298:1233–1236
- Krumholz LA (1948) Reproduction in the western mosquitofish, *Gambusia affinis affinis*, and its use in mosquito control. *Ecol Monogr* 18:1–43
- Lawler SP (1989) Behavioral responses to predators and predation risk in four species of larval anurans. *Anim Behav* 38:1039–1047
- Lever C (1996) *Naturalized fishes of the world*. Academic, New York
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Littell RG, Stroup GA, Walter W, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary, NC
- Lodge DM (1993) Biological invasions: lessons for ecology. *Trends Ecol Evol* 8:133–137
- Lonsdale WM (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology* 80:1522–1536
- Lydeard C, Wooten MC, Meyer A (1995) Molecules, morphology, and area cladograms: a cladistic and biogeographic analysis of *Gambusia* (Teleostei: Poeciliidae). *Syst Biol* 44:221–236
- Mack RN (1996) Predicting the identity and fate of plan invaders: emergent and emerging approaches. *Biol Conserv* 78:107–121
- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl* 10:689–710
- Margaritora FG, Ferrara O, Vagaggini D (2001) Predatory impact of the mosquitofish (*Gambusia holbrooki* Girard) on zooplanktonic populations in a pond at Tenuta di Castelporziano (Rome, Central Italy). *J Limnol* 60:189–193
- Maron JL, Vila M (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95:36–373
- McPeck MA (1990) Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. *Ecology* 71:1714–1726

- Meffe GK (1985) Predation and species replacement in American southwestern fishes: a case study. *Southwest Nat* 30:173–187
- Milinski M, Heller R (1978) Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.). *Nature* 275:642–644
- Miller RG (1981) Simultaneous statistical inference. Springer, Berlin Heidelberg New York
- Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. *Nature* 421:625–627
- Moyle PB (2002) Inland fishes of California. University of California Press, Berkeley, CA
- Moyle PB, Light T (1996) Biological invasions of freshwater: empirical rules and assembly theory. *Biol Conserv* 78:149–161
- Myers GS (1965) *Gambusia* the fish destroyer. *Trop Fish Hobbyist* 13:31–32, 53–54
- Ogutu-Ohwayo R (1999) Nile perch in Lake Victoria: balancing the costs and benefits of aliens. In: Sandlund OT, Schei PJ, Viken A (eds) Invasive species and biodiversity management. Kluwer, Dordrecht, pp 47–63
- Petren K, Case TJ (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77:118–132
- Quinn JL, Cresswell W (2004) Predator hunting behaviour and prey vulnerability. *J Anim Ecol* 73:143–154
- Rehage JS (2003) Traits underlying invasiveness: A comparison of widespread and endemic species in the genus *Gambusia* (Poeciliidae). Dissertation. University of Kentucky, Lexington, KY
- Rehage JS, Sih A (2004) Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biol Invasions* 6:379–391
- Reusch TBH (1998) Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in Southern California. *Mar Ecol Prog Ser* 170:159–168
- Ricciardi A, Atkinson SK (2004) Distinctiveness magnifies the impact of biological invaders in aquatic systems. *Ecol Lett* 7:781–784
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thomson JN, Weller SG (2001) The population biology of invasive species. *Annu Rev Ecol Syst* 32:305–332
- Schlaepfer MA, Runge MC, Sherman PW (2002) Ecological and evolutionary traps. *Trends Ecol Evol* 17:474–480
- Schoenherr AA (1981) The role of competition in the replacement of native fishes by introduced species. In: Naiman RJ, Soltz DL (eds) Fishes in North American deserts. Wiley, New York, pp 173–203
- Scribner KT (1993) Hybrid zone dynamics are influenced by genotype-specific variation in life-history traits: experimental evidence from hybridizing *Gambusia* species. *Evolution* 47:632–646
- Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. *Trends Ecol Evol* 17:170–176
- Sih A (1982) Foraging strategies and the avoidance of predation by an aquatic insect, *Notonecta hoffmanni*. *Ecology* 63:786–796
- Sih A (1987) Predators and prey lifestyles: an evolutionary and ecological overview. In: Kerfoot WC, Sih A (eds) Predation: direct and indirect impacts on aquatic communities, University Press of New England, Hanover, MA, pp 203–224
- Sol D, Lefebvre L (2000) Behavioral flexibility predicts invasion success in birds introduced to New Zealand. *Oikos* 90:599–605
- Sol D, Timmermans S, Lefebvre L (2002) Behavioral flexibility and invasion success in birds. *Anim Behav* 63:495–502
- Stachowicz JJ, Whitlatch RB, Osman RW (1999) Species diversity and invasion resistance in a marine ecosystem. *Science* 286:1577–1579
- Stohlgren TJ, Binkley D, Chong GW, Kalkhan MA, Schell LD, Bull KA, Otsuki Y, Newman G, Bashkin M, Son Y (1999) Exotic plant species invade hot spots of plant diversity. *Ecol Monogr* 69:25–46
- Tilman D (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78:81–92
- Tilman D (1999) The ecological consequences of change in biodiversity: a search for general principles. *Ecology* 80:1455–1474
- Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. *Nature* 421:628–630
- Tsutsui ND, Suarez AV, Grosberg RK (2003) Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proc Natl Acad Sci* 100:1078–1083
- Underwood AJ (1997) Experiments in ecology. Cambridge University Press, Cambridge
- USFWS (1996) San Marcos and Comal Springs and associated aquatic ecosystems recovery plan. U.S. Fish and Wildlife Service, Austin, TX
- Usher MB (1988) Biological invasion of nature reserves: a search for generalizations. *Biol Conserv* 44:119–135
- Usio N, Konishi M, Nakano S (2001) Species displacement between an introduced and a 'vulnerable' crayfish: the role of aggressive interactions and shelter competition. *Biol Invasions* 3:179–185
- Vermeij GJ (1996) An agenda for invasion biology. *Biol Conserv* 78:3–9
- Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. *Am Sci* 84:468–479
- Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *NZ J Ecol* 21:1–16
- Webb C, Joss J (1997) Does predation by the fish *Gambusia holbrooki* contribute to declining frog populations? *Aust Zool* 30:316–324
- Williamson M (1996) Biological invasions. Population and Community Biology Series 15. Chapman and Hall, London
- Winemiller KO, Taylor DH (1987) Predatory behavior and competition among laboratory-housed largemouth and smallmouth bass. *Am Midl Nat* 117:148–166
- Zane L, Nelson WS, Jones AG, Avise JC (1999) Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *J Evol Biol* 12:61–69